those of the F.S.H. group which gave no response (or only an attenuated response) while members of Series I, promptly gave a full or an increased response when later injected with prolactin. Similarly those rats of Series I which had failed to respond to prolactin dosage were reprimed and later injected with an *unfractionated* pituitary extract (10 mg. daily) whose prolactin content was at least 1 unit per mg. To the 13th day of such injection no rat that failed to respond to prolactin had responded to the administration of the several pituitary hormones.

Summary. In virgin rats aged 67-81 days, with ovaries stimulated by Prolan or F.S.H. administration during 5 days, the injection of purified and previously heated prolactin induced maternal behavior in 6 of 10 rats after 6-10 injections. Previous conditioning by concaveation was limited to a single 12-hour period in 4 of these rats. The maternal instinct, in somewhat varying degrees, was exhibited by all these rats while the daily subcutaneous injections were in progress. Six of 7 rats of the control and F.S.H. injected groups, all showing little or no maternal behavior during a 25-day test, later developed or greatly accentuated their maternal behavior after 1 to 8 days treatment with prolactin. Four rats which showed no maternal behavior under prolactin also later failed to show such behavior after repriming and 13 days of administration of unfractionated pituitary extract. Prolactin is the anterior pituitary hormone specifically concerned in the activation of maternal behavior in virgin rats.

7838 P

Effect of Dietary Fats on Growth and Composition of Tumors.

FRANCES L. HAVEN. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

While many studies of the effect of diet on tumor growth have been made, few deal with the quality of the dietary fat and its effect on the lipid content of the tumor. Sugiura and Benedict[1, 2] found that oral administration of 10% cod liver, olive, linseed and chaulmoogra oils had no influence upon susceptibility to and growth of

[1] Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1930, **14**, 311.

[2] Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1925, **9**, 204.

Flexner-Jobling rat carcinoma. Feeding 40% butter fat gave a decreased take and growth rate of Flexner-Jobling rat carcinoma, and increased regressions. The same diet had no influence upon the growth of a rat sarcoma. Freund, Lustig and Kellner[3] found that diet free from palmitin but rich in olein rendered half of a series of mice refractory to carcinoma inoculation, and caused a decreased carcinoma growth rate in the animals. Caspari[4] reported retarded growth of tumors in mice fed large amounts of butter fat or palmitin.

This report deals with the effect of a synthetic diet, varying only in the nature of the fat, on the growth of rat Carcino-Sarcoma No. 256 obtained from Dr. Frances Wood of Columbia University. The rats were inoculated subcutaneously in the groin by the trocar method. On the day of inoculation, they were placed on Diet 262, Sinclair.[5]

From 4 to 7 weeks after inoculation, the rat was decapitated, the tumor removed, weighed, and portions of the outside taken for analysis. Cholesterol, fatty acids, and phospholipids were determined by Bloor's method[6, 7] and the micro iodine numbers by Yasuda's modification[9] of the Rosenmund-Kuhnhenn[8] method.

The growth of the tumor is expressed by a formula of Bierich and Lang[10] involving the weight of the tumor in grams and its age in days from the time of inoculation, or $Wt = a \cdot t^2$; a (growth tendency of tumor) $= \frac{\text{weight}}{\text{time}} \times 10{,}000$. The growth tendencies of rat tumors on cocoanut oil diet (Iodine No. = 9) have been found to be larger than those of rat tumors on cod liver oil diet (Iodine No. = 157) (Table I).

In 4 experiments, Table I, the cocoanut and cod liver oil animals were inoculated with the same tumor and placed on the respective diets the day of inoculation. Similar results were obtained in one experiment in which menhaden oil, a fish body oil of Iodine No. 175, was substituted for cod liver oil.

Since cocoanut and cod liver oil differ so widely in iodine number it seemed best to compare the iodine numbers of the tumor phospholipid fatty acids. Table II shows results on cocoanut, men-

[3] Freund, E., Lustig, B., and Kellner, B., *Z. Krebsforsch.*, 1932, **37**, 355.

[4] Caspari, W., *Z. Krebsforsch.*, 1933, **38**, 355.

[5] Sinclair, R. G., *J. Biol. Chem.*, 1931, **92**, 245.

[6] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[8] Rosenmund, K. W., and Kuhnhenn, W., *Z. Untersuch. Nahr.—Genussm*, 1923, **46**, 154.

[9] Yasuda, M., *J. Biol. Chem.*, 1931, **94**, 401.

[10] Bierich, R., and Lang, A., *Z. physiol. Chem.*, 1933, **216**, 217.

TABLE I.
Comparative Growth Tendency (a) of Rat Carcino-Sarcoma 256 on Cocoanut Oil and Cod Liver Oil Diets.

Experiment	1	2	3	4
Cocoanut Oil (I.N. 9)	108	97	114	144
	132	105	115	171
	138	121	139	202
	185	123	139	
	192		147	
			153	
			152	
			154	
			194	
Av. (21 animals) = 144	151	112	145	172
Cod Liver Oil (I.N. 157)	72	60	78	55
	86	86	107	104
	123	88	166	117
	135	92		163
		93		202
Av. (17 animals) = 107	104	84	117	128

haden and cod liver oil diets with a few on a fat-poor diet; Diet 3, Sinclair.[5]

TABLE II.
Influence of Food Fat on Degree of Unsaturation (Iodine Numbers) of Phospholipid Fatty Acids of Rat Carcino-Sarcoma No. 256.

Diet	Fat-Poor	Cocoanut Oil	Cod Liver Oil	Menhaden Oil
Determinations	6	17	18	8
Av.	84±2.3	94±3.1	106±4.8	112±2.1

Although Hartwell,[14] and Harris and Moore[15, 16] have shown that the incorporation of 12-15% cod liver oil in the diet inhibits the growth of rats, the retardation of tumor growth apparently is not due to such an effect. Sinclair (private communication to the author) has shown the growth curves of uninoculated rats on the cod liver oil diet used, to be essentially the same as those on the cocoanut oil diet, and normal Moreover, in the 21 rats on the cocoanut oil diet, the weights of the rats at death minus the weights of the tumors averaged 176 gm. while those of the 17 cod liver oil rats averaged 179 gm.

The inhibitory action of unsaturated fats on the growth of tumors can be explained by the theory that phospholipids function as oxygen transport agents within the cell.[11] The respiration of tumor cells is

[14] Hartwell, G. A., *Biochem. J.*, 1927, **21**, 1076.
[15] Harris, L. J., and Moore, T., *Biochem. J.*, 1928, **22**, 1461.
[16] Harris, L. J., and Moore, T., *Biochem. J.*, 1930, **23**, 1114.
[11] Sinclair, R. G., *Physiol. Rev.*, 1934, **14**, 351.

unusual. The incorporation of unsaturated fatty acids into the phospholipids of tumor cells may aid ordinary respiration as opposed to glycolysis, thus inhibiting tumor growth.

The iodine numbers of the phospholipid fatty acids of this tumor are low compared with those of muscle on the same diets. Sinclair[5] found for muscle: fat-poor diet—101; cocoanut oil diet—124; and cod liver oil diet—160; with menhaden oil probably in the range of cod liver oil values. There are several plausible explanations for these low iodine numbers. In the first place, the long chain unsaturated fatty acids of the diet may not enter the tumor which, being a carcino-sarcoma, consists of epithelium and connective tissue. Possibly connective tissue does not take up the unsaturated acids of the diet. A study of a pure carcinoma and sarcoma, comparing effects of these diets on similar rat tissues may settle this point.

In the second place, the ratio of saturated to unsaturated fatty acids in the tumor may be different from that in muscle. Determinations of amounts of solid and liquid fatty acids using the lead soap method, will clarify this point.

In the third place, the unsaturated fatty acids of the diet may enter the tumor phospholipids but be immediately reduced or saturated. Boyland[12] and Harris[13] have shown that tumors contain some as yet unidentified reducing substance in addition to glutathione and ascorbic acid. It is not known whether this substance reduces unsaturated fatty acids.

7839 C

Reaction to Differentiate Vitamin A from Carotene by Means of Antimony Trichloride.*

ALFRED C. ANDERSEN AND VICTOR E. LEVINE.

From the Department of Biological Chemistry and Nutrition, School of Medicine, Creighton University, Omaha, Nebraska.

The Carr-Price reaction for vitamin A and carotene consists in the development in chloroform solution of a characteristic blue color

[12] Boyland, E., *Biochem. J.*, 1933, **27**, 802.

[13] Harris, L. J., *Nature*, 1933, **132**, 605.

* The carotene used in our work was obtained from the S.M.A. Corporation of Cleveland, Ohio, and consists largely of β-carotene with small amounts of α-carotene.